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GRADUATE RESEARCH ARTICLE

Determination of Heavy Metals in Root and Shoot of Durum Wheat, Plant Soil: Translocation and Bioaccumulation Factor

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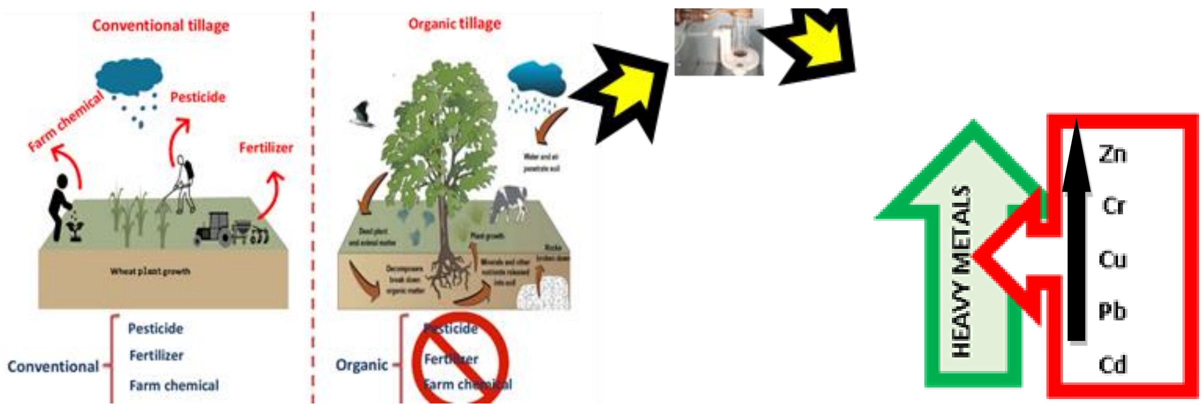
ABSTRACT

Heavy metals are uptake by the soil, transfer and accumulate in the various parts of durum wheat plant. The exposure of wheat crops to heavy metals poses threat to their physiological growth and human health through consumption. The study aimed at finding the concentration of heavy metals in durum wheat varieties, transfer and bioaccumulation ability, and effect of organic and conventional farming on heavy metals. The wheat samples (under organic and conventional farming) were randomly sampled at tillering, jointing, and grain filling stage. They were digested with ultra-microwave and analyzed with inductively coupled plasma. The results showed that Zn emerged as the highest concentration in the soil, root, and shoot of all wheat samples and mostly followed by Cr, Cu, Pb, and Cd. With few instances, an order of Cu>Zn>Cd>Cr>Pb>As translocation and accumulation was observed. Statistical study (α .05 and CI of 95%) revealed conventional farming increased heavy metals in the soil. Therefore, organic farming should be adopted to prevent heavy metal contamination of wheat plants and higher food chain.

Keywords: Heavy metals, Translocation and bioaccumulation factor, Durum wheat



1 Introduction

Wheat cereal is consumed in many countries due to its rich content in vitamins, minerals, and proteins [1]. Wheat comes in a variety of species and two most often grown are hard wheat (*Triticum durum* or durum wheat) with high protein content and used for pasta products and soft wheat (*Triticum aestivum*) used for breakfast foods due to low protein content [2]. Durum wheat is a stable food and grown mainly in the Mediterranean basin supported by Italy as one of the producers [3]. Durum wheat is mostly refined into semolina powder which is used for pasta and other bakery products due to its enriched nutrients. The quality of the wheat products depends mostly on the environmental conditions, genetic variability, and cultivation methods of the wheat plant [4]. Like most plants, wheat is at the base of the food chain and susceptible to contaminations from the environment, both organic and inorganic (heavy metals) contaminants which are uptake by the soil and plants and can be detrimental to human health after consumption. Wheat has a tendency to absorb and accumulate heavy metals from the soil and also atmospheric deposition on foliar surfaces through the stomata; Pb deposition on wheat crops has been reported [5]. The exposure to even low concentration of heavy metals can affect soil organisms negatively and higher up the food chain. Therefore, previous research has focused on the transfer and accumulation of heavy metals by the soil-crop system with higher Zn accumulation in wheat grain [6]; soil due to anthropogenic activities such as the use of agrochemicals, and wastewater for irrigation [7]; and higher transfer from soil to plants with Cr, Ni, and Pb exceeding the WHO safe limit by irrigating the crops with untreated water [8]. Heavy metals affect the normal physiological growth of the crops and decrease its biomass and quality [9]. Therefore, several phytoremediation techniques to remediate heavy metals from the soil-crop system has been an ongoing research, with the use of foliar technology to reduce the content of arsenic and lead by the application of silicon to the wheat crops [9]; phytoextraction; phytostabilization; phytovolatilization; phytofiltration; phytotransformation; and rhizodegradation have also been employed to eliminate the content of heavy metals [10]. Despite the consequences of heavy metals enumerated so far, several essential heavy metals, such as Cu, Fe, Mn, Co, Zn, and Ni, are required by plants at low concentrations due to their functions as cofactors that are essential for the structural and functional integrity of enzymes and other proteins [11]. A problem exists if the concentration of the essential elements are above the normal threshold for the plant growth. Another alternative approach to reduce the content of heavy metals in crops is the use of organic farming method. Organic cultivation action utilizes organic fertilizers, compost, and indigenous methods to provide plants with nutrients; the conventional method focuses on chemicals to provide nutrients to the plants. The application of organic fertilizer obtained from animal waste to vegetable crops resulted in a 3.21%-62.28% yield higher than the conventional practice with 2.18%-24.34% yield [12], and higher plant height, number of seeds and yield of wheat crops [13]. Another report was published on 5.35% yield in wheat and other cereals by the adoption of integrative nanofertilizers of nitrogen and zinc with compost compared to the conventional practice, thus confirming the advantages of this farming practice [14]. In addition, due to less toxicity to crops and environment, natural products from organic farming are more patronized by consumers compared to the conventional method [15]. Despite the advantages of the aforementioned farming practice, there are reports on mixed effects on crops and soil. Organic treatment increased the content of lead, arsenic, cadmium, and pesticides in soil [12]. Therefore, emerging issues on whether organic method is healthier than the conventional method is an ongoing research. Nevertheless, the advantages of organic method are numerous in the literature with further report of providing soil microorganisms with rich food source [16]. Owing to the aforementioned matters, the current work was planned to investigate whether organic cultivation is more advantageous compared to conventional cultivation in heavy metals uptake in durum wheat crops and the following objectives were considered: (1) to determine some heavy metals (Cr, Hg, Cu, Zn, As, Cd, Pb) present in durum soil-wheat system using inductively coupled plasma (ICP) and X-ray fluorescence (XRF); (2) to determine the translocation and bioaccumulation factors of the heavy metals under study; (3) to compare the effect of heavy metals in organic and conventional farming system. The results of this research will be beneficial to the agricultural and food industry.

2 Materials and Methods

2.1 Chemicals, Reagents and Standard Solutions

Ultra Scientific Analytical Standard (IQC-026) containing 26 analytes (Zn, Al, Tl, Ti, Se, Ni, Mo, Mn, Mg, Pb, Fe, Cu, Co, Cr, Ca, Cd, Be, As, Sb, Na, Ag, Si, K, B, Ba, and Va), with concentration of 100 pm for each was obtained from Analisi Control Srl laboratory and used for the calibration curve; nitric acid and grade 1 and 2 water. The matrix of the standard is water mixed with diluted tartaric and nitric acids. All chemicals were of analytical grade.

2.2 Crop cultivation and pest control

The wheat durum plants were grown under organic and conventional cultivation methods (Saragolla, Fuego, Evoldur) with few exceptions (Senatore Cappelli, Saragolla Antico and San Carlo) which were grown under only organic and conventional method respectively (Table 1). Ammonium nitrate fertilizer (148 kg/ha) and (304 kg/ha) was applied to the crops cultivated under conventional farming to supply nutrients to the crops and weed control was performed using Amadeus Top (50 g/ha) dicotyl. + Traxos Pronto (1 lt/ha) which is a common herbicide for soft and hard durum wheat. For organic cultivation, Guanito (200 kg/ha) natural organic fertilizer was used for pre-treatment of the seed before planting, and DIX N10 (400 kg/ha) organic fertilizer to further enhance the nutrients availability.

2.3 Study site and sampling

The samples were obtained from Jesi, in the province of Ancona in Marche region, Italy (Figure 1). The area has a linear increase in temperature during the day from 6 °C to 16 °C and a windy climate. The sampling was carried out at upper end of the plant (20 cm above the ground) and at different growth stages (tillering stage, jointing stage, and grain filling stage) (Table 1). The samples were kept in plastic polythene container, sealed, and transported to the laboratory at 4 °C. The wheat plants were washed severely with distilled and ultra-pure water to remove dirt and contaminants which might affect the analysis and kept refrigerated at 4 °C until sample preparation.

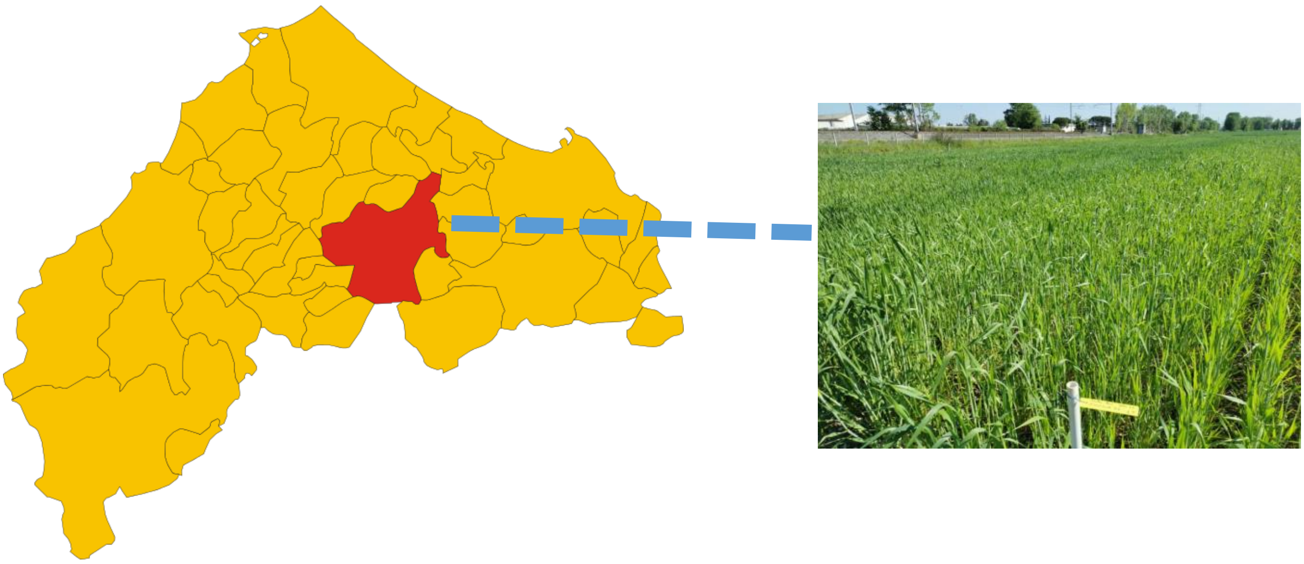


Figure 1: *Map of province of Ancona (Yellow). Jesi, where samples for the study were obtained (red)*

Table 1: *Sampling of durum wheat varieties in Jesi study area*

ID CODE	SAMPLE DESCRIPTION	ID CODE	SAMPLE DESCRIPTION
SOIL		**PLANT**	
Soil1_1M	SARAGOLLA durum wheat[1]	**Plant1_1M**	SARAGOLLA durum wheat[1]
Soil1_2M	SARAGOLLA durum wheat[2]	**Plant1_1M**	SARAGOLLA durum wheat[2]
Soil2_1M	SAN CARLO durum wheat[1]	**Plant2_1M**	SAN CARLO durum wheat[1]
Soil3_1M	FUEGO durum wheat[1]	**Plant3_1M**	FUEGO durum wheat[1]
Soil3_2M	FUEGO durum wheat[2]	**Plant3_1M**	FUEGO durum wheat[2]
Soil4_1M	EVOLDUR durum wheat[1]	**Plant4_1M**	EVOLDUR durum wheat[1]
Soil4_2M	EVOLDUR durum wheat[2]	**Plant4_1M**	EVOLDUR durum wheat[2]
Soil5_2M	SENATORE CAPPELLI durum wheat[2]	**Plant5_1M**	SENATORE CAPPELLI durum wheat[2]
Soil6_2M	SARAGOLLA ANTICO durum wheat[2]	**Plant6_1M**	SARAGOLLA ANTICO durum wheat[2]
Soil7_2M	FUEGO durum wheat[2] ▪	**Plant7_1M**	FUEGO durum wheat[2] ▪

[1]*Conventional cultivation;* [2]*Organic cultivation;* ▪*Near railway*

The sampling of the wheat plant was carried out at the upper end of the plant (20 cm above the ground). The plant material was divided into root and shoot and analyzed separately.

2.4 Sample preparation of wheat crops and soil

The samples were recovered from the refrigerator and dried in Argo lab TCN 200 oven at 55 °C for 24 hours to eliminate the moisture. After removal of visible organic matter and debris and passing through 2-mm sieve, the wheat soil was crushed into smaller size and the root and shoot blended separately; all samples were stored at room temperature in a plastic polythene container with their appropriate labelling. Approximately 0.5 g of the wheat root, shoot, and soil samples were weighed into a glass tube and 4 ml HNO_3 (65%) and 1 ml HCL (37%) were added. The mixture was allowed under the fume hood for about 5 minutes for initial digestion to occur. Then, it was digested using Ultra-wave Microwave digester (Table 2 and Figure S1) at the aforementioned laboratory for 40 minutes. The digested samples were allowed under the fume hood for few minutes to ensure complete digestion, cooling of the glass tubes and release of acid vapour. Then, the samples were recovered in a 50 ml vial with several rinsing, labelled and kept at 4 °C until further analysis. Grade 1 water was used in all preparations.

Table 2: *Microwave operation parameters used for the digestion of the samples*

Nr	t	MW [W]	T1 [°C]	T2 [°C]	P1 [bar]
1	00:25:00	1500	230	70	120.0
2	00:10:00	1500	230	70	120.0

Nr (stages). t (time). MW (microwave power). T1 (temperature in microwave reactor). T2 (temperature in reactor ring). P1 (pressure in reactor).

2.5 X-ray fluorescence analysis of samples

The samples were homogenized using Retsch® ball and mill homogenizer at University of Camerino at 500 rpm for 1 hour. The X-ray analysis was carried out only for the grain filling stage with Saragolla and Fuego samples. The homogenized samples were recovered in plastic containers, covered with paraffin film, and kept at room temperature prior to analysis. Analysis was carried out using Shimadzu Energy Dispersion Fluorescence X-ray Spectrometer, Rayny Series EDX-800HS2 CE (Table S1) at the aforementioned laboratory. A total of 200 seconds was used for the analysis of each tablet sample; 100 seconds for scanning from the line Ti-U and Na-Sc.

2.6 Inductively coupled plasma (ICP) analysis of samples

The samples were analyzed using Thermo Scientific ICAP PRO at the aforementioned laboratory. An external calibration standards (Cr, Hg, Cu, Zn, As, Cd, Pb) were prepared with concentrations of 10, 1, 0.1, 0.01, and 0.001 ppm using serial dilution method from the analytical standard. In brief, 10 ppm working standard was prepared by diluting 1 ml of the stock standard to 10 ml volumetric flask. This working standard solution was used to prepare the remaining solutions. For analysis of mercury, a separate standard with concentrations of 1, 0.1, 0.01, and 0.001 ppm was prepared due to the problem of instability with other elements in the sample solution. The sample solutions were filtered using a 0.45 µm RC Sartorius filter and injected for analysis. In each case, analysis was carried out in triplicate. Grade 2 water and 1% HNO_3 were used in all dilutions during the preparations.

2.7 Statistical analysis

Excel software was used to prepare bar charts, and column charts to visualize the data. Due to the small size of the samples, Wilcoxon signed-rank test, a non-parametric test, was used to check for the null hypothesis of the concentration of the metals in the conventional and organic cultivation practices. The transfer (TF) and bioaccumulation (BCF) factors were calculated for the root and shoot as TF =Shoot/Root, and BCF (root) = Root/Soil, BCF (shoot) =Shoot/Soil respectively [17].

3 Results and Discussion

3.1 Analysis of Heavy metals in soil samples

The results from tillering and jointing stage are presented in this paper in mg/kg (Figure 2) and (Table S2 and S3). No results are presented from X-ray fluorescence analysis due to less sensitivity to detect the heavy metals under study. The results from grain filling stage can be found in the supplementary (Figure S2). Our results showed that Zn emerged as the metal with the highest concentration in all the soils used for the cultivation of the durum wheat varieties under conventional and organic processes, followed by Cr, consistent with the results reported by Gupta *et al.*, (2022)[18]. The negative impact of excessive Zn in soil enzymes and microorganisms must be mentioned here [19]. However, this study showed a safe limit of Zn in the cultivated soil, thus exhibiting less threat to crops [20]. It is worth noting that all the wheat cultivars tested under conventional and organic conditions exhibited the lowest quantities of Cd, As, and Pb [21] compared to the background values of agricultural soil in Italy [22]; Hg and As were below the detection threshold. Similarly, Setia *et al.*, (2022) found comparable results for lowest values of Cd, and As in wheat soil [23], but in contrast with Tagumira *et al.*, (2022), who reported Cd content higher than the restrictions of the European Union [24]. Lead is one of the toxic elements and can affect the reproductive system by interacting with biomolecules [25]. Fortunately, the level is tolerable in the cultivated soil in this work. In this study, the conventional method had higher Zn, Cu, Cr, and Pb concentrations compared to the organic method, with few exception (Fuego durum wheat) [26]. A comparable report has been published [27] with more pronounced effect of conventional farming to the organic method in the cultivation soil. In contrast, higher content of Cd, Cu, Zn, Pb, Cr, and As have been reported under organic compared to conventional farming [12]. However, it should be noted that their analysis was based on soil used for cultivating vegetables and the excessive application of organic fertilizer; thus explaining the variability in the results. Generally, an order of Zn>Cr>Cu>Pb>Cd>As>Hg was observed in this study in the cultivated soil and were within the allowed limit for soil set by the Italian Ministry [22].

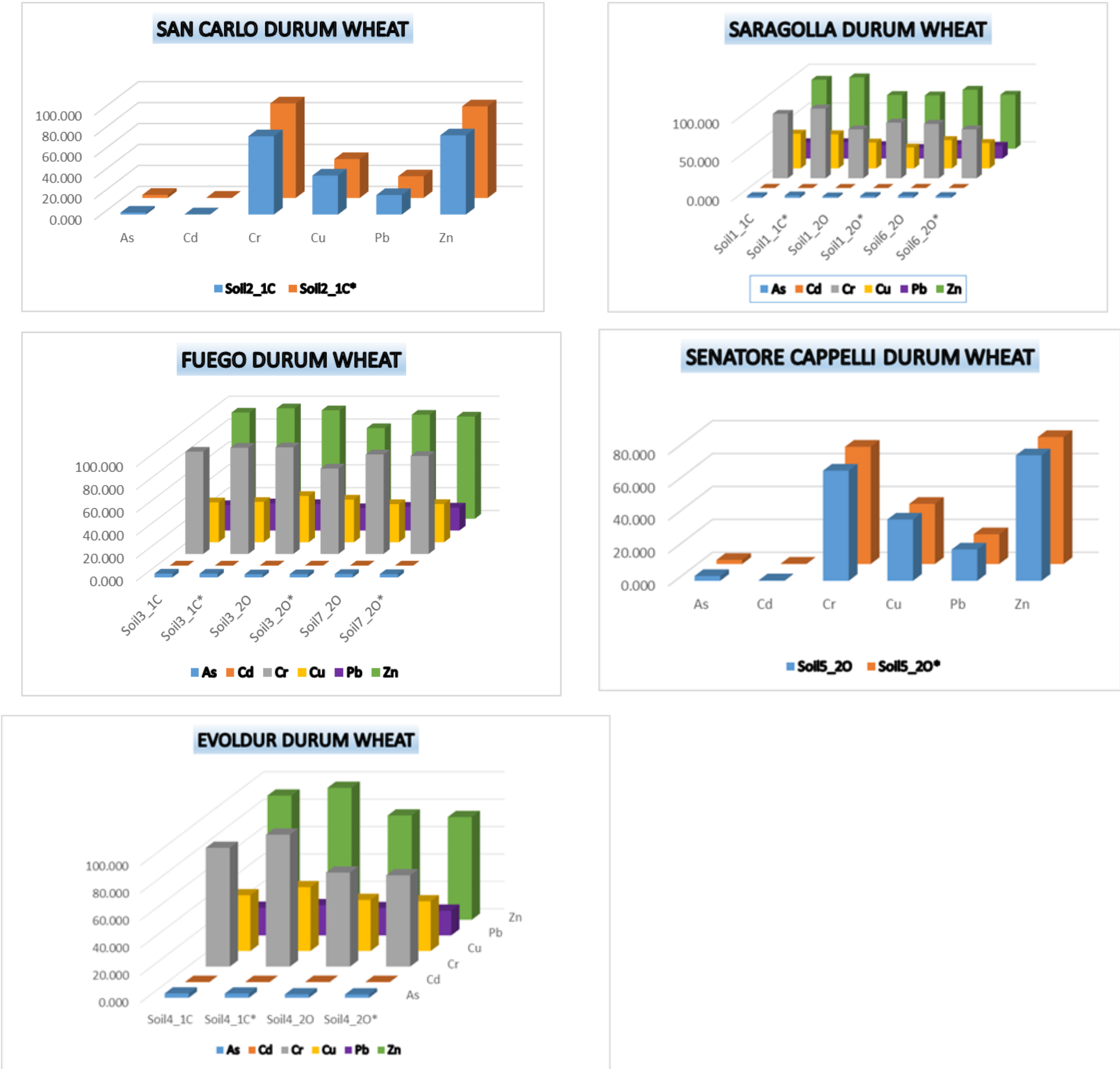


Figure 2: *Mean concentration (triplicate analysis) of heavy metals (mg/kg) in Soil of durum wheat varieties. C (Conventional farming practice). O (Organic farming practice). *Tillering stage*

3.2 Analysis of Heavy metals in shoot and root samples

Zn remained the metal with a higher mean concentration in the shoot in all wheat types under both cultivation methods (Figure 3) and (Table S4 and S5) followed by Cu; a comparable result was observed in the root (Figure 4) and (Table S6 and S7). The position of Cu and Cr interchanged compared to the soil samples. Among the shoot and root, the latter presented higher content of heavy metals consistent with the published data [23], [28]. The root has close proximity to the soil as opposed to the aerial portions of the plant, increasing the exposure to heavy metals. In contrast to the soil samples, an order of Zn>Cu>Cr>Pb was observed for the shoot samples [21]. It is worth noting that both the root and shoot recorded a low content of metals compared to the soil suggesting poor transfer ability. In another study, a contrasting report was published with higher content of the metals in the crops than the soil [24]. The variability can be explained that their crops were some cereals other than wheat. The type of plant species is an important factor that affects the transfer of substances from the soil to the crop [21].

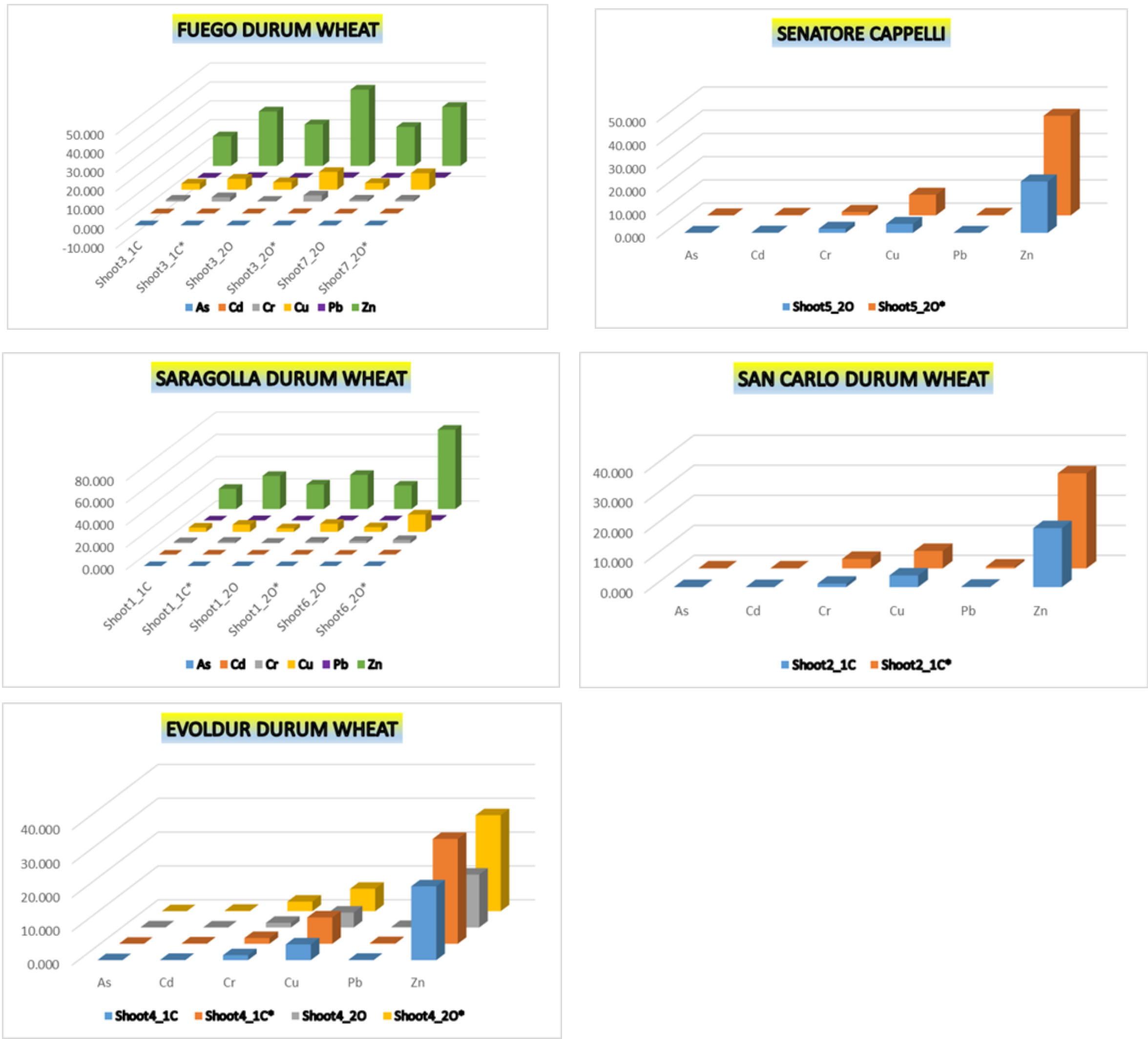


Figure 3: *Mean concentration (triplicate analysis) of heavy metals (mg/kg) in Shoot of durum wheat varieties. C (Conventional farming practice). O (Organic farming practice). *Tillering stage.*

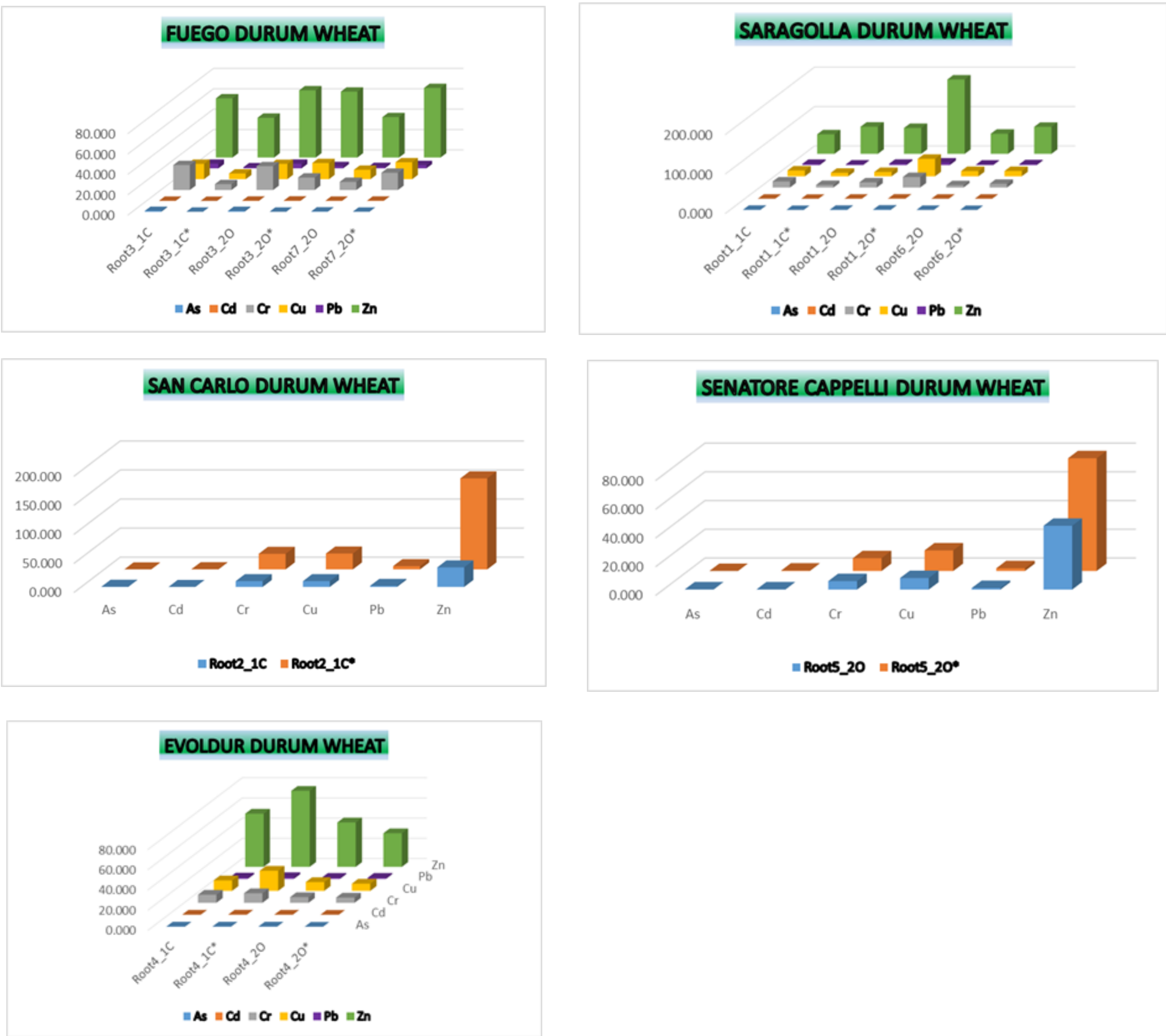


Figure 4: *Mean concentration (triplicate analysis) of heavy metals (mg/kg) in Root of durum wheat varieties. C (Conventional farming practice). O (Organic farming practice). *Tillering stage.*

3.3 Accumulation and Translocation factor of the Heavy metals

The most transported metal from root to shoot was Cu, followed by Zn, and Cd in the wheat plant (Figure 5). The results showed that the transfer factor was less than 1 for most metals except Cu in a few case indicating the mobility from the soil to root and shoot. This means that the shoot acts as a storage organ for these metals. Generally, an order of Cu > Zn > Cd > Cr > Pb was observed for the transfer ability. Both Zn and Cu are essential elements required in moderate quantity by plants and thus readily transferred to the plant tissues [29]. A comparable study reported that Zn and Cu were easily transferred into the wheat plant organs [6], thus consistent with the current findings. From figure 6 and 7, we provide an evidence that Zn was more accumulated in the root from the soil followed by Cd and a regular pattern of Zn>Cd>Cu>Cr>Pb was mostly observed. However, it should be noted that Cd was more accumulated in the shoot from the soil. This is consistent with the work of Rezapour *et al.*, (2019) [30] who reported Cd accumulation was higher in the wheat root. The results published by Setia *et al.*, (2022) agrees with the current findings as Zn was highly accumulated in the wheat crop than the other metals [31]. Accumulation of heavy metals in the soil-crop system is affected by physiological and environmental factors which is associated with their availability in the soil [6]. However, the transfer and accumulation of heavy metals are affected by several factors including the kind of heavy metal, pH, temperature, organic matter, soil texture, solubility, and type of plant species [32]. A plant may absorb a metal in the root, but presents poor distribution in the other aerial parts. The absorption and transfer depends heavily on the exchangeable and dissolved form of the metal ions.

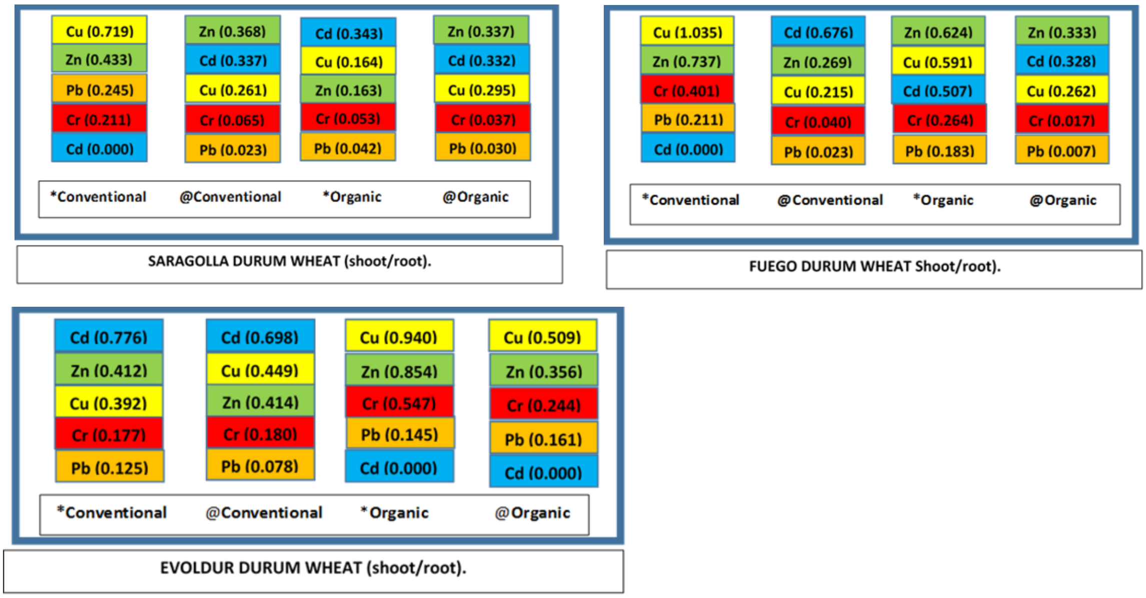


Figure 5: *Translocation factor from root to shoot of Saragolla, Fuego, and Evoldur durum wheat. *Tillering stage. @Jointing stage.*

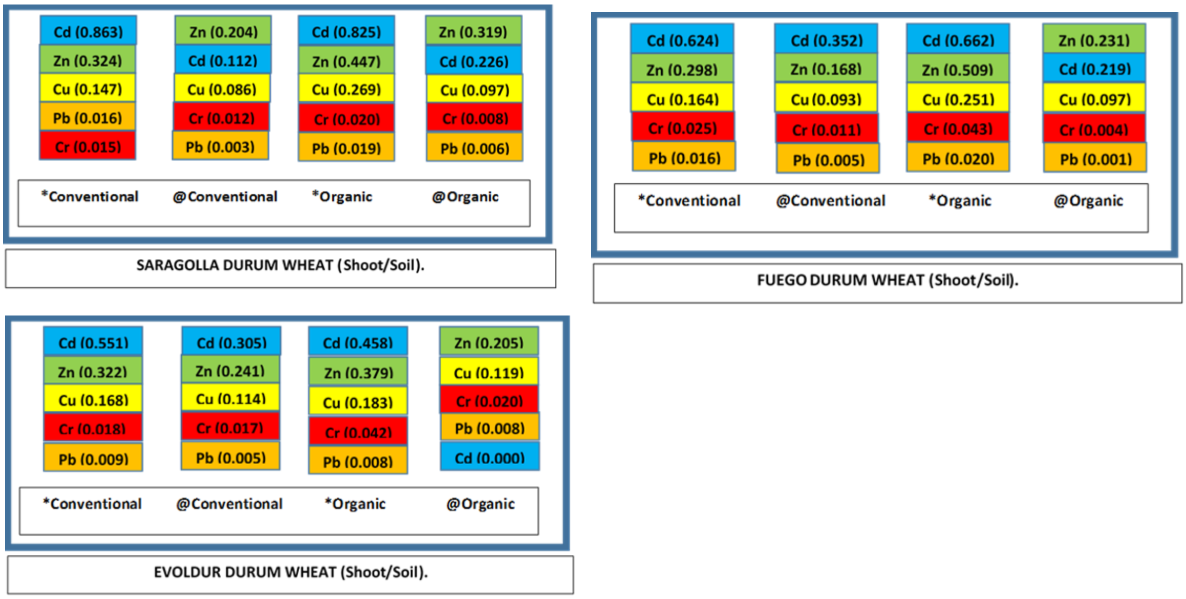


Figure 6: *Bioaccumulation factor from soil to shoot of Saragolla, Fuego, and Evoldur durum wheat. *Tillering stage. @Jointing stage.*

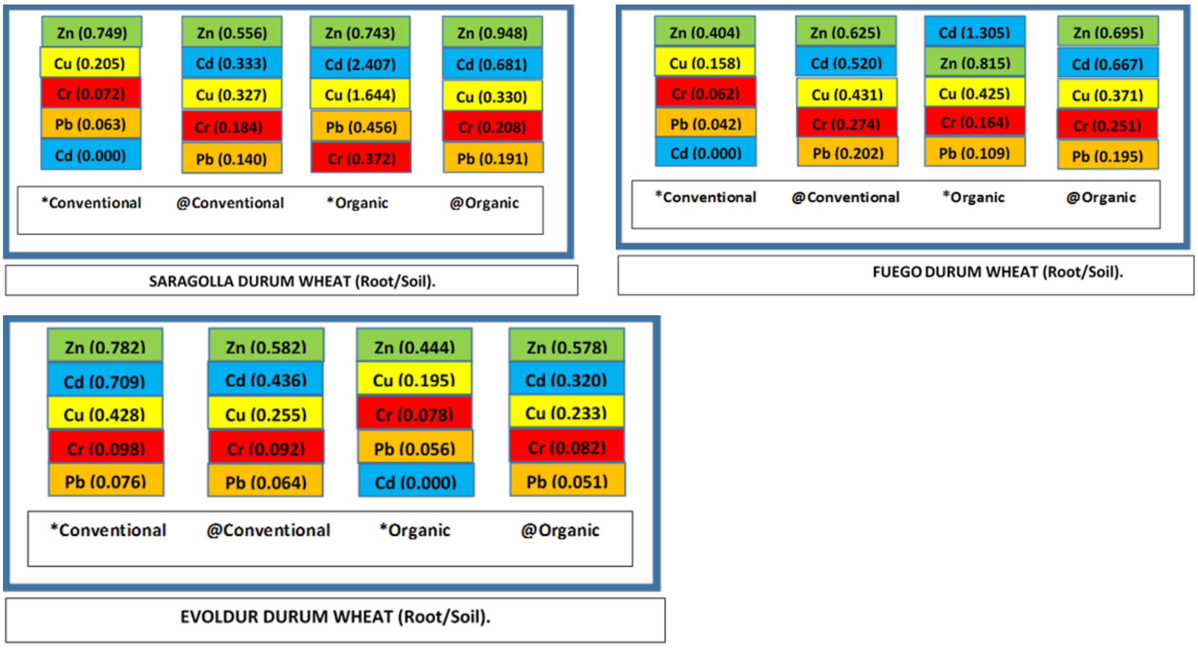


Figure 7: *Bioaccumulation factor from soil to root of Saragolla, Fuego, and Evoldur durum wheat. *Tillering stage. @Jointing stage.*

3.4 Effect of heavy metals in conventional and organic cultivation method

Table 3 presents the summary of the Wilcoxon signed-rank test which was conducted at a significance level of 0.05 and confidence interval of 95%.

Table 3: *Summary of tests on total metals. O (Organic). C (Conventional).*

Stage	Sample	Wheat variety	p-value	Null hypothesis*
Tillering	Soil1_2O Soil1_1C	Saragolla	0.028	Reject
	Soil3_2O, Soil3_1C	Fuego	0.173	Retain
	Soil4_2O, Soil4_1C	Evoldur	0.046	Reject
Jointing	Soil1_2-2O, Soil1_1-2C	Saragolla	0.028	Reject
	Soil3_2-2O, Soil3_1-2C	Fuego	0.075	Retain
	Soil4_2-2O, Soil4_1-2C	Evoldur	0.046	Reject
Tillering	Root1_2O Soil1_1C	Saragolla	0.028	Reject
	Root3_2O, Soil3_1C	Fuego	0.043	Reject
	Root4_2O, Soil4_1C	Evoldur	0.028	Reject
Jointing	Root1_2O Soil1_1C	Saragolla	0.753	Retain
	Root3_2O, Soil3_1C	Fuego	0.750	Retain
	Root4_2O, Soil4_1C	Evoldur	0.116	Retain

*Differences of median between variables of organic and conventional cultivation are the same. The statistical test was conducted at a significance level of 0.05 and confidence interval of 95%.

The results showed a statistically significant in Saragolla, and Evoldur cultivars, and insignificant in Fuego cultivar (soil). For shoot, statistically insignificant for all wheat varieties. For root, statistically significant for all wheat varieties (Tillering), and insignificant (Jointing stage). Thus, the statistical analysis agrees with the fact that the conventional method introduced more availability of metals in the soil and root as compared to the shoot. This agrees with the concentration of the heavy metals in the cultivated soil reported in this work. In the work of Zaccone *et al.*, (2010), wheat samples were cultivated under organic and conventional farming system without compost and organic system with compost. The study showed a higher effect of the heavy metals under the conventional system, consistent with this work [33], but contrast the published work [34] where the content of Cr, Cd, and Pb were higher under organic cultivation method. In fact, the reason for the demand for organic foods by consumers is the absence or presence of low chemical substances, thus advocating human health [35]. In contrast, some studies have reported the tendency of organic farming system to promote significant pollutants in the soil due to long-term application of manure and compost, especially when used untreated [12], [36]. Thus, the quantity of organic fertilizers applied to the soil and crop should be monitored regularly. On the other hand, conventional system has been reported to promote higher yield [37], especially to accommodate the growing demand for food, but excessive application can damage the environment, crops and affect human health. However, the work by Albano *et al.*, (2023) showed the potential of organic system to promote wheat crop yield [38]. In their work, organic amendments of winter wheat promoted crop yield than inorganic fertilizer application. Therefore, the use of organic farming system with effective pest control and treatment of the manure substance is the optimum practice to achieve better yield and more healthy food.

4 Conclusion

The present study indicated that the heavy metals were abundant in the soil compared to the root and shoot of the wheat plants. Zn was abundant in the soil, root and shoot among the metals studied in this work with Cd at the lowest level. Hg and as were below the detection threshold. Apart from few cases, an order of Cu > Zn > Cd > Cr > Pb > As translocation and accumulation was observed indicating the possibility of these metals to be transferred into the wheat plants. The statistical study (α .05 and CI of 95%) showed conventional farming promoted the availability of heavy metals in the soil and root compared to organic farming. Therefore, the results of this study contribute to the knowledge on wheat crop cultivation. Quantification of organic fertilizers applied to crops and effective pest control method is the key to better yield and quality foods in the market.

5 Declarations

5.1 Competing Interests

The authors declare no conflict of interests.

5.2 Publisher's Note

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Supplementary File

Supplementary file with tables and figures is available at https://journals.aijr.org/index.php/ajgr/article/view/7180/572

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